510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k042476

B. Purpose for Submission:

New submission

C. Measurand:

Valproic Acid

D. Type of Test:

Quantitative Immunoassay with calibrators and controls

E. Applicant:

Ortho-Clinical Diagnostics, INC.

F. Proprietary and Established Names:

Vitros Chemistry Products VALP Reagent

Vitros Chemistry Products Calibrator Kit 12

Vitros Chemistry Products TDM Performance Verifiers I, II and III

G. Regulatory Information:

1. Regulation section:

   862.3645 Neuroleptic drugs radioreceptor assay test system
   862.3200 Calibrators, Drug Specific
   862.3280 Clinical Toxicology Control Material

2. Classification:

   Class II, II, I (Reserved)

3. Product code:

LEG, DLJ, DIF respectively

4. Panel:

   91 (Toxicology)

H. Intended Use:

1. Intended use(s):

   See indications for use.

2. Indication(s) for use:

   VITROS Chemistry Products VALP Reagent is used to quantitatively measure valproic acid in human serum and plasma. Serum or plasma valproic acid measurements are used in the diagnosis and treatment of valproic acid overdose and in monitoring levels of valproic acid to ensure appropriate therapy. VITROS Chemistry Products Calibrator Kit 12 is used to calibrate VITROS 5,1 FS Chemistry System for the quantitative measurement of valproic acid (VALP). VITROS Chemistry Products TDM Performance Verifier is an assayed control used to monitor performance on VITROS Chemistry Systems.

   The device is for in vitro diagnostic use.

3. Special conditions for use statement(s):

   The assay is for prescription use.
   The assay is not designated for use in point-of-care settings.

4. Special instrument requirements:
   Vitros 5,1 FS Chemistry Analyzer. (k031924)

I. Device Description:

The valproic acid reagent consists of a dual chambered package containing two liquid ready-to-use reagents that are used in a two-step reaction.

Reagent 1 consists of mouse monoclonal antibodies to valproic acid, NAD, and glucose-6-phosphate (G-6-P). Reagent 2 consists of valproic acid labeled with glucose-6-phosphate dehydrogenase (G-6 PD). When combined, valproic acid in the sample competes with labeled valproic acid for antibody sites. G-6-PD activity is decreased when it is bound to the antibody. The resulting conversion of NAD to NADH in the reaction is measured spectrophotometrically at 340 nm.

Calibrator Kit 12 are standards that are sold separately. They are used to construct

the standard curve, which is used to calculate the concentration of the unknown samples. They consist of 6 levels of aqueous solution containing valproic acid, ranging in concentration from 0-150 µg/mL.

Verifiers I, II and III are a set of 3 assayed controls (6 vials- 2 mL each) that are run with the samples to monitor the performance of the assay. They consist of bovine serum spiked with valproic acid.

Diluent Pack 2 is comprised of two chambers, one containing water and salts, and the other 7% BSA. It is used to dilute samples on the analyzer.

Human Source Material: The sponsor indicates there are no human source materials in their product.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   SYVA Emit 2000 Valproic Acid Assay and Calibrators

   TDM Performance Verifiers currently in commercial distribution

2. Predicate 510(k) number(s):

   k002551 and k953197, respectively

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative measurement of valproic acid.	Quantitative measurement of valproic acid
Basic principle	Homogeneous enzyme immunoassay	Homogeneous enzyme immunoassay
Reportable range	10-150 µg/mL	1-150 µg/mL
Sample type	Serum and plasma	Serum and plasma

Differences		
Item	Device	Predicate
Instrumentation	VITROS 5,1 FS Chemistry System	SYVA-30R Biochemical System

K. Standard/Guidance Document Referenced (if applicable):

The sponsor referenced the following guidance document(s) in their submission:

National Academy of Clinical Biochemistry Symposium. Standards of Laboratory practice: antiepileptic drug monitoring. Clinical Chemistry 44:5 1085-1095 (1998).

NCCLS document EP9-A. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline- Second Edition.

NCCLS document EP5-A. Method Comparison of Precision Performance of Clinical Chemistry Devices; Approved Guideline.

NCCLS document EP7-A. Interference Testing in Clinical Chemistry; Approved Guideline.

NCCLS document EP6-A. Evaluation of the Linearity of Quantitative Measurement Procedure: A Statistical Approach; Approved Guideline.

The sponsor does not indicate any deviation from these guidance documents.

L. Test Principle:

The test is an enzyme immunoassay for use on the Vitros 5,1 FS Chemistry System. Calibrators, ranging in concentration from 0 to150 µg/mL, are run with the assay. The Vitros VALP assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of valproic acid in human serum and plasma. In the performance of the Vitros VALP assay, serum or plasma is mixed with Reagent 1 which contains mouse monoclonal antibodies to valproic acid and the coenzyme nicotinamide adenine dinucleotide (NAD). Subsequently, Reagent 2, containing Valproic acid labeled with glucose-6-phosphate dehydrogenase (G6PDH), is added. Valproic acid in the sample and the valproic acid labeled G6PDH compete for the antibody binding sites. Enzyme activity decreases upon binding to the antibody, so valproic acid concentration in the sample can be measured in terms of enzyme activity. Enzyme activity converts NAD+ to NADH, resulting in an absorbance change that is measured spectrophotometrically at 340nm. Unknowns are calculated from a standard curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The samples used for testing were the sponsor's controls, Verifiers I, II and III. The samples were run in duplicate, twice a day for twenty-two days using three lots and three instruments.
The sponsor states that studies were conducted according to NCCLS Guidelines (EP5-A) for precision.

The results of the test samples were analyzed by ANOVA as detailed in NCCLS EP5-A. A nested ANOVA was performed to estimate within-day, day-to-day, week-to-week (cal-to-cal) and total within-lab precision.

The within-day and within-laboratory precision is summarized in the table below.

VITROS Chemistry Products VALP Reagent Precision

SYSTEM	Conventional Units (µg/mL)			SI Units (µmol/L)			Within Lab %CV	No. Observations	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**	Mean Conc.	Within Day SD*	Within Lab SD**			
VITROS 5,1 FS	22.55	0.71	1.31	156.3	4.9	9.1	5.8%	88	22
	61.25	1.62	2.83	424.4	11.3	19.6	4.6%	88	22
	103.41	2.30	3.80	716.6	15.9	26.3	3.7%	88	22

*Within Day precision was determined using two runs / day with two replications per run.
** Within Lab precision was determined using a single lot of reagents and calibrating weekly.

b. Linearity/assay reportable range:

To evaluate the linearity the sponsor compared the calculated and measured valproic acid concentrations for a series of test fluids that span the reportable range of the assay. The sponsor states that studies were performed according to NCCLS Guideline EP6-A8, Evaluation of the Linearity of Quantitative Analytical Methods.

Linearity fluids were prepared from two pools of serum with valproic acid near the extremes of the calibration range (low pool 3µg/mL and high pool 170 µg/mL). The two pools were mixed to create 15 additional pools of intermediate concentrations.

Five determinations of each linearity fluid level and three determinations of Verifiers were tested with three lots of reagent and two analyzers. A linear regression analysis was performed by the method of least squares. The plotted

curve conforms to a straight line, supporting the reportable range 10.0-150.0 µg/mL.

Dilution Study:
The sponsor conducted a dilution study to verify recommended diluent performance and verify the accuracy of results using diluted specimens.

2 Lots of reagent were used. Dilutions (X2 and X4) were performed on board by the analyzer.

Six frozen human serum samples with valproic acid concentrations near the upper end or above the reportable range (concentrations ranging from 113 to 136 µg/mL) were tested. Results for four of the undiluted samples were within the reportable range of the analyzer (10.0 – 150.0 µg/mL). Results for two of the undiluted samples were above the reportable range of the analyzer. Each neat and corresponding diluted sample pair was tested in triplicate and a mean was calculated for the neat sample and the diluted sample.

The mean diluted sample value was divided by the mean undiluted sample value and then multiplied by 100 to determine percent recovery. The % recovery of all samples ranged from 90-103%.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Calibrators and controls are required with this assay and are specifically identified in the labeling. They are both sold separately, and are obtaining clearance with this submission. See the device description section, above.

Traceability is achieved in the value assignment process through the use of U.S. Pharmacopeia standard valproic acid, catalog NO. 1708707. Standards (primary calibrators) are gravimetrically prepared by the manufacturer and six levels are confirmed by GC/MS or HPLC methods. To assign values to the manufacturer's working calibrator the sponsor first calibrates the SYVA test system using the primary calibrators. One hundred human clinical serum samples are assigned values from this run. The serum samples are then used to calibrate the VITROS test system, and to assign values to the sponsors' master lot of calibrators. This procedure ensures that matrix effects associated with both methods are taken into account in the value transfer process. The sponsor's master lot is used to assign all subsequent lots of product.

Control values are assigned by running five Vitros 5,1 FS Chemistry System in five different laboratories using manufactures products. Data was collected over 10 days, 2 runs/day and 2 replicates/run for each performance verifier.

STABILITY
Shelf life and open vial stability was established by running human serum

aliquots at concentrations of 19 µg/mL, 75 µg/mL and 119 µg/mL. Each sample pool was run at baseline (time = 0) and at 1, 2, 3, 7, 9, 13, 15, and 19 months. At each test interval 1 bottle of each level was used to calibrate a Syva 30R Immunodiagnostic analyzer. 20 replicates of each pool were tested to assess variations in the concentration over time. The mean of each testing material for valproic acid was then calculated and compared to the acceptance criteria.

Acceptance Criteria:

The acceptance limits are based on the valproic acid concentrations:
If the valproic acid concentration is < 28 µg/mL, the limit is ± 2.363 µg/mL. If the valproic acid concentration is ≥ 28 µg/mL, the limit is calculated using the following equation:

0.0448 [valproic acid] + 1.1382 µg/mL

All data met the acceptance criteria except for pool 2 and 3 at 9 months. Subsequent data points collected at 13, 15 and 19 months passed, therefore, the data supports an 18-month expiry.

d. Detection limit:

To determine the lower limit of detection the sponsor evaluated serum from ten normal adult human donors (containing no valproic acid) and their zero calibrator. Each of the samples was analyzed in triplicate.

The 12 samples were analyzed six times, using three reagent lots and two lots of the calibrator on two instruments.

The data were analyzed for each of the six determinations to calculate the lower limit of detection (LLD) as shown:

$$\text{Pooled Replicate SD} = \sqrt{\sum_{i=1}^{j}(n_i - 1)*(SD_i)^2 \Big/ \sum_{i=1}^{j}(n_i - 1)}$$

$$\text{LLD} = \text{Mean zero calibrator} + (\text{Pooled Replicate SD} \times 3)$$

All the lower limit of detection estimates ranged from 2.7 µg/mL to 2.9 µg/mL.
The data support the product claim for a lower limit of detection of 2.9 µg/mL.

e. Analytical specificity:

Eleven human serum samples having valproic acid concentrations of approximately 70 µg/mL were pooled and designated as "low" pool. Twelve human samples had approximately 130 µg/mL of valproic acid and were designated as the "high" pool. These samples were used in testing the interference of bilirubin and hemoglobin.

Twelve human serum samples having valproic acid concentrations of approximately 70 µg/mL were pooled and designated as "low" pool. Nine human serum samples having valproic acid concentrations of approximately 130 µg/mL were pooled and designated as "high"pool. These samples were used in testing the interference of Intralipids.

A serum base pool containing approximately 70 µg/mL valproic acid was used in testing all remaining test substances.

The sponsor indicates that they followed the protocol outlined in NCCLS document EP7-A Interference Testing In Clinical Chemistry for paired-difference method.

Four determinations of each pool containing a particular test substance were made using two reagent lots on one VITROS 5,1 FS Chemistry System. For each substance tested, the mean valproic acid concentration, SD and CV (%) were calculated for each control pool and the test substance pool.

Bias was calculated as:
Bias = Mean conc. of test substance pool - Mean conc. of control pool

The % cross-reactivity was calculated as:

$$\% \text{ Cross-Reactivity} = \frac{\text{Mean conc. with substance} - \text{Mean conc. w/o substance}}{\text{Conc. of substance}} \times 100$$

The predetermined acceptance criteria are acceptance limits based on the valproic acid concentrations in the control pool. The limits are calculated using the following equation:

0.0696(valproic acid) + 1.7685 µg/mL

None of the substances tested generated a bias greater then $\pm$7%.

The substances listed in this table, at the concentrations shown, were tested according to NCCLS Protocol EP7-A with VITROS VALP Reagent and a serum pool at a valproic acid concentration of 70 µg/mL, and found not to interfere [bias <6.6 µg/mL]. Bilirubin, hemoglobin and Intralipid were also

tested with a serum pool at a valproic acid concentration of 135 µg/mL, and found not to interfere [bias < 11.2 µg/mL].

A serum pool at a valproic acid concentration of 70 µg/mL the substances were found not to interfere (bias < 6.6 µg/mL). Bilirubin, Hemoglobin and Intralipid were tested in a serum pool at a valproic acid concentration of 135 µg/mL and found not to interfere (bias < 11.2 µg/mL).

Compound	**Concentration**	
Bilirubin	60 mg/dL	1026 µmol/L
Carbamazepine	1000 µg/mL	4.2 mmol/L
Clonazepam	100 µg/mL	317 µmol/L
Diazepam	100 µg/mL	351 µmol/L
Ethosuximide	1000 µg/mL	7.1 mmol/L
Hemoglobin	1000 mg/dL	10 g/L
Intralipid	1000 mg/dL	10 g/L
2-n-Propyl-3-hydroxy-pentanoic acid	100 µg/mL	624 µmol/L
2-n-Propyl-4-hydroxy-pentanoic acid	100 µg/mL	624 µmol/L
2-n-Propyl-5-hydroxy-pentanoic acid	50 µg/mL	312 µmol/L
2-n-Propyl-3-oxo-pentanoic acid	100 µg/mL	633 µmol/L
Phenobarbital	750 µg/mL	3.2 mmol/L
Phenytoin	1000 µg/mL	4.0 mmol/L
Primidone	1000 µg/mL	4.6 mmol/L
2-Propyl glutaric acid	400 µg/mL	1.6 mmol/L
2-Propyl-2-pentenoic acid	20 µg/mL	141 µmol/L
2-Propyl-4-pentenoic acid	10 µg/mL	703 µmol/L
2-Propyl succinic acid	500 µg/mL	3.1 mmol/L

Absorbance values exceeding 3.0 AU were observed with the VALP Reagent with the "high" serum containing valproic acid at approximately 130 µg/mL spiked with Intralipid at levels of 800 mg/dL or higher. When these samples were tested after dilution with 7% BSA (1 part 7% BSA and 1 part sample) valproic acid results were reported and all levels of Intralipid tested passed acceptance limits. No interferences were found.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor states that studies were based on NCCLS Guideline EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples*.

A total of 96 serum samples were assayed by the predicate and candidate device. Each sample was measured in triplicate on each system. Testing was performed with three lots of reagents on three VITROS Systems. The range of samples tested was 14.3-147.1 µg/mL.

The relationship between the two methods, determined by least squares linear regression is:

Y= 0.97(vitros) + 1.3 µg/mL
Syx = 4.24

b. Matrix comparison:

To determine which specimen types are suitable for analysis with the assay, the sponsor conducted a study involving various specimens from different BectonDickinson tubes.

Blood samples from multiple individuals were drawn into serum (red top) collection tubes. Valproic acid in either a methanol matrix or a human serum base matrix were "spiked" into the samples to a concentration of 50, 100 and 200 µg/mL, mixed and transferred to various tube types. The following table shows specimen types and fill levels for the specimen examined. The quarter filled tubes were evaluated to determine the effects of under-filled conditions.

Sample Type	**Fill Levels Collected**
Serum (Red Top)	Full
Serum Separator (SST)	¼ Full
Li-Heparin Plasma	Full, ¼ Full
Li-Heparin Plasma Separator (PST)	Full, ¼ Full
Sodium Citrate	Full, ¼ Full
EDTA Plasma	Full, ¼ Full
Sodium Fluoride Potassium Oxalate	Full, ¼ Full

Serum and plasma samples were evaluated by paired-difference testing. All samples were tested in triplicate with one lot of reagent.

The data were tabulated and the bias between the mean value (n=3) for each test condition was compared to the mean of the serum value. The bias was calculated as:

Bias = Test Condition Value – Serum Sample Value

Serum (red top) was used as the reference because it is the specimen matrix used to establish overall accuracy of the method.

The acceptance criteria are based on the valproic acid concentration in the serum sample :

Valproic Acid Concentration (µg/mL)	Bias Limits	% Bias Limits
50	± 5.25 µg/mL	± 10.5 %
100	± 8.73 µg/mL	± 8.7 %
200	± 15.69 µg/mL	±7.8 %

The bias between serum values (from a red top tube) and values from the serum separator tubes (SST) and from the anticoagulants EDTA and lithium heparin (including plasma separator tubes) all met the acceptance criteria.

The bias values observed between serum samples and samples collected in tubes containing Sodium Fluoride Potassium Oxalate or Sodium Citrate did **not** meet acceptance criteria.

3. Clinical studies:

   a. Clinical Sensitivity:
   Not applicable.

   b. Clinical specificity:
   Not applicable.

   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.

4. Clinical cut-off:
   Not applicable.

5. Expected values/Reference range:

   Reference ranges are presented, below.

Classification	Conventional Units (µg/mL)	SI Units (µmol/L)	Alternate Units (mg/L)
Minimal	50.0	346.5	50.0
Therapeutic	50.0–120.0	346.5-831.6	50.0-120.0
Possible toxic	>100.0	693.0	>100.0
Serious Toxic	>200.0	1386.0	>200.0

These ranges are the same reference ranges cited by the predicate device. Use of the same ranges is supported by the close agreement observed in the linear regression plot from the method comparison study.

N. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.